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PENDING CLAIMS

Listing of claims:

1 (Currently amended). A method for the preparation of macrocyclic molecules comprising:

contacting a purified ~~excised~~ protein consisting of a thioesterase (TE) domain selected from a polyketide synthase (PKS) or a non-ribosomal peptide synthetase (NRPS) ~~thioesterase (TE) domain protein~~ with a substrate for said ~~purified excised~~ thioesterase (TE) domain ~~protein~~ that comprises an activated acyl residue and a pendant nucleophile separated by a linear backbone under conditions conducive to formation of a TE-O-acyl bond such that subsequently the pendant intramolecular nucleophile can displace the TE domain to form the macrocyclic product.

2 (Currently amended). A macrocyclization method as in claim 1 wherein the contacting of the ~~excised TE domain~~ protein with a substrate occurs in a medium that comprises at least 90 % water.

3 (Currently amended). A macrocyclization method as in claim 2, wherein the contacting of the ~~excised TE domain~~ protein with a substrate occurs in a medium that comprises at least 95 % water.

4 (Currently amended). A macrocyclization method as in claim 2, further comprising ~~wherein the~~ non-water component(s) wherein the non-water component(s) is an organic solvent having a sulfoxide, ester, or amide functional group.

5 (Currently amended). A macrocyclization method as in claim 1, wherein the contacting of the ~~excised TE domain~~ protein with a substrate occurs in an aqueous solution comprising one or more buffers or other organic or inorganic salts.

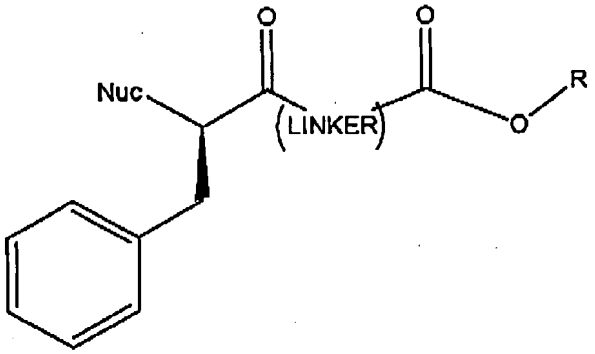
6 (Previously Presented). A macrocyclization method as in claim 1, wherein the pH of the reaction solution is in the range of 5 to 9.

7 (Previously Presented). A macrocyclization method as in claim 6, wherein the pH of the reaction solution is in the range of 6 to 8.

8 (Previously Presented). A macrocyclization method as in claim 6, wherein the pH of the reaction solution is 7.

9 (Original). A macrocyclization method as in claim 1, wherein the activated acyl residue is an activated ester functional group.

10 (Previously Presented). A macrocyclization method as in claim 9, wherein the substrate can be represented by the formula:

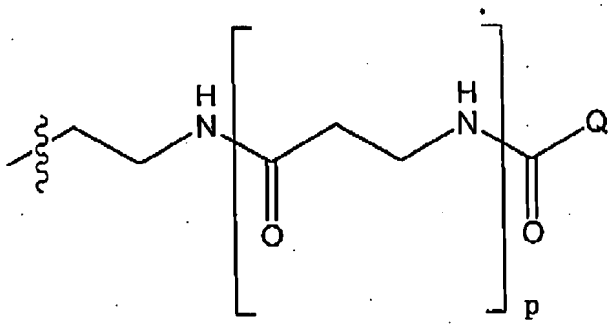


wherein

Nuc is chosen from NH_2, OH or SH;

LINKER is a peptidic sequence, a synthetic group comprising alkyl, cycloalkyl, alkenyl, alkynyl, aryl groups or a group that comprises a combination of two or more alkyl, cycloalkyl, alkenyl, alkynyl or aryl group regions and 0 to 3 heteroatoms selected from N, O, and S, or a combination thereof connecting the ester and the 2-(Nuc)-3-phenyl-propionyl residue, the LINKER comprises a linear backbone of at least 14 atoms; and

R is a group that can be represented by the formula:

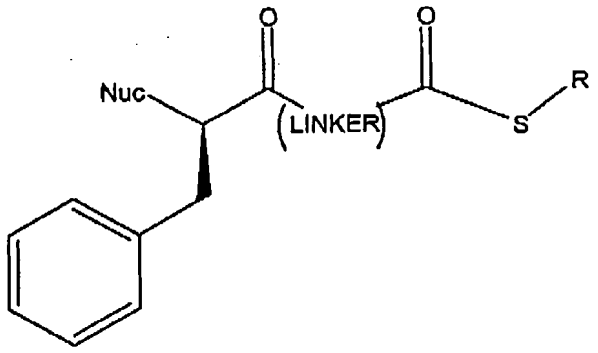


wherein Q is a group having between 4 carbon atoms and 20 carbon atoms and between 0 and 10 hetero atoms selected from N, O or S, which can optionally be tethered to a solid support, where each carbon of the linear backbone may be optionally substituted with 0, 1, or 2 groups selected from C_{1-6}alkyl, hydroxy, amino, halogen, C_{1-6}alkoxy, or oxo; and

p is an integer from 0 to 2.

11 (Original). A macrocyclization method as in claim 1, wherein the activated acyl residue is an activated thioester functional group.

12 (Previously presented). A macrocyclization method as in claim 11, wherein the substrate can be represented by the formula:



wherein:

Nuc is chosen from NH_2, OH or SH;

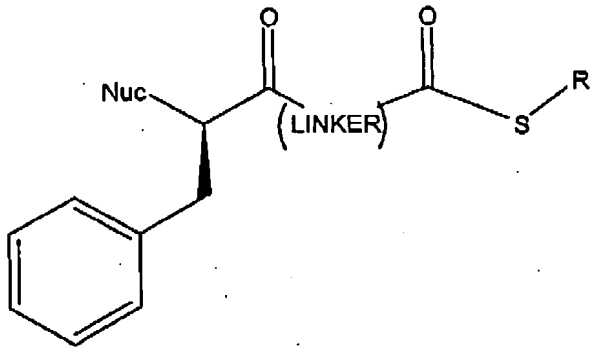
LINKER is a peptidic sequence, a synthetic group comprising alkyl, cycloalkyl, alkenyl, alkynyl, aryl groups or a group that comprises a combination of two

or more alkyl, cycloalkyl, alkenyl, alkynyl or aryl group regions and 0 to 3 heteroatoms selected from N, O, and S, or a combination thereof connecting the thioester and the 2-(Nuc)-3-phenyl-propionyl residue, the LINKER comprises a linear backbone of at least 14 atoms; and

R is an optionally substituted C_{1-12} alkyl group or an optionally substituted N-C_{2-6}alkanoyl-C_{2-6}aminoalkyl group.

13 (Original). A macrocyclization method as in claim 12, wherein the substrate is sufficiently polar such that its solubility and that of the resulting macrocycle molecule in the aqueous reaction medium is at least 0.1 g/L.

14 (Previously presented). A macrocyclization method as in claim 11 , wherein the substrate can be represented by the formula:



wherein:

Nuc is chosen from NH_2, OH or SH;

LINKER is a peptidic sequence, a synthetic group comprising alkyl, cycloalkyl, alkenyl, alkynyl, aryl groups or a group that comprises a combination of two or more alkyl, cycloalkyl, alkenyl, alkynyl or aryl group regions and 0 to 3 heteroatoms selected from N, O, and S, or a combination thereof connecting the thioester and the 2-(Nuc)-3-phenyl-propionyl residue, the LINKER comprises a linear backbone of at least 14 atoms; and

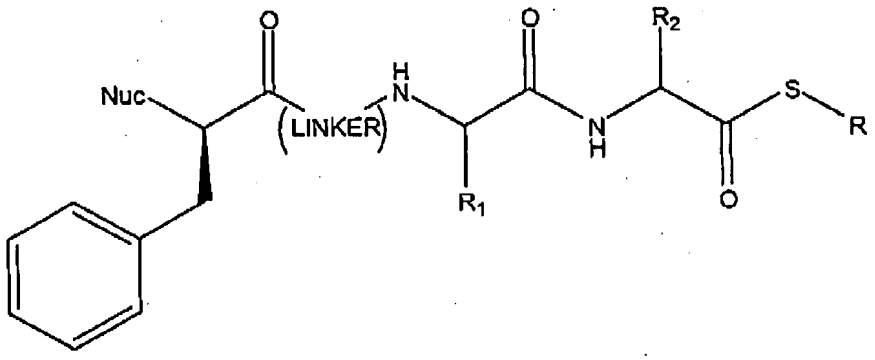
R is a N-C_{2-6}alkanoyl-C_{2-6}aminoalkyl.

15 (Original). A macrocyclization method as in claim 14, wherein the substrate leaving group, SR, is N-acetylcysteamine (SNAC).

16 (Original). A macrocyclization method as in claim 12, wherein Nuc is NH_2.

17 (Original). A macrocyclization method as in claim 12, wherein Nuc is OH.

18 (Previously Presented). A macrocyclization method as in claim 12, wherein the substrate can be represented by the formula:



wherein

Nuc is chosen from NH_2, OH or SH;

LINKER is a peptidic sequence, a synthetic group comprising alkyl, cycloalkyl, alkenyl, alkynyl, aryl groups or a group that comprises a combination of two or more alkyl, cycloalkyl, alkenyl, alkynyl or aryl group regions and 0 to 3 heteroatoms selected from N, O, and S, or a combination thereof connecting the thioester and the 2-(Nuc)-3-phenyl-propionyl residue, the LINKER comprises a linear backbone of at least 6 atoms;

R is as defined for Claim 12; and

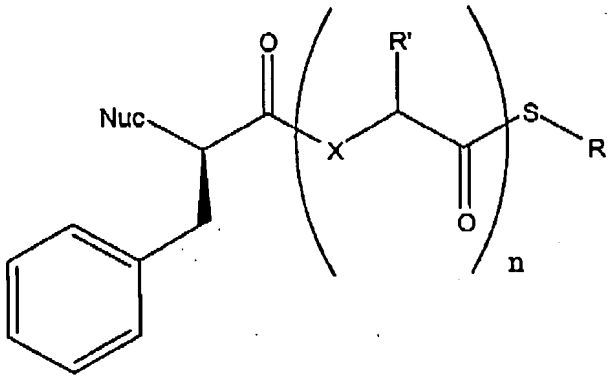
R_1 and R_2 are chosen from the side chain substituents of a synthetic and biosynthetic amino acid residue side chains and each residue can have either D or L stereoconfiguration, R_1 and R_2 are chosen independently and can be the same or different.

19 (Original). A macrocyclization method as in claim 18, wherein the substrate is sufficiently polar such that its solubility and that of the resulting macrocyclic molecule in the aqueous reaction medium is at least 0.1 g/L.

20 (Previously presented). A macrocyclization method as in claim 18, wherein R_1 is a synthetic or biosynthetic amino acid residue side chain substituent including a substituted C_{1-6}aminoalkyl group.

21 (Original). A macrocyclization method as in claim 20, wherein R_1 is L-3-aminopropyl.

22 (Previously presented). A macrocyclization method as in claim 11, wherein the substrate can be represented by the formula:



wherein:

Nuc is chosen from NH_2 or OH;

n is an integral number greater than or equal to 5;

X is independently chosen from O and NH for each occurrence of X;

R is an optionally substituted N-C_{2-6}alkanoyl-C_{2-6}aminoalkyl;

R' is independently chosen for each occurrence for R' from the side chain substituents of the synthetic and biosynthetic amino acid residue side chains and each amino acid residue can have either D or L stereoconfiguration.

23 (Original). A macrocyclization method as in claim 22, wherein:

Nuc is NH_2; and

X is NH for each occurrence of X in the substrate.

24 (Original). A macrocyclization method as in claim 22, wherein:

Nuc is NH_2; and

X is chosen from O and NH for each occurrence of X in the substrate such that at least one occurrence of X in the substrate is O.

25 (Original). A macrocyclization method as in claim 22, wherein:

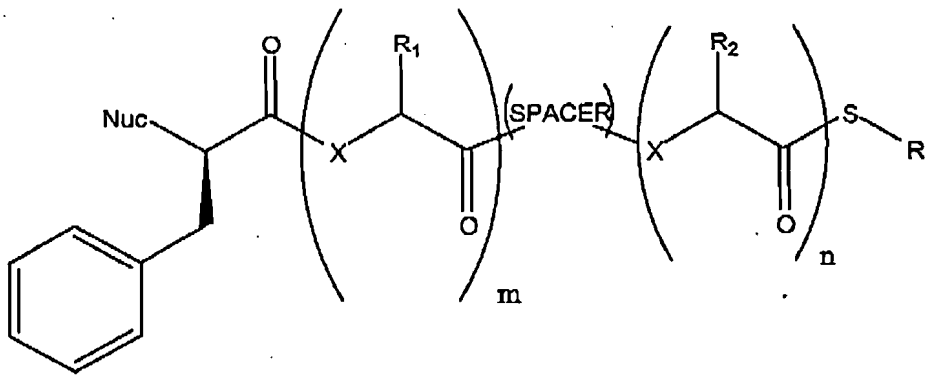
Nuc is OH; and

X is NH for each occurrence of X in the substrate.

26 (Previously Presented). A method as in claim 22, wherein n is between 5 and 15 inclusive.

27 (Original). A method as in claim 22, wherein at least one occurrence of R' is 3-aminopropyl.

28 (Previously Presented). A macrocyclization method according to claim 12, wherein the substrate that comprises at least one non-peptidic spacer can be represented by the formula:



wherein:

Nuc is chosen from NH_2 or OH;

m and n are non-negative integers;

X is independently chosen for each occurrence of X in the formula to be either O or NH;

SPACER is a group of atoms or functional group residues that are not amino acid residues or depsi residues that comprise z atoms in the linear backbone of the substrate;

z is an integral number greater than or equal to 4;

the sum of z + 3m + 3n is between 12 and 36; and

R_1 and R_2 are chosen from the side chain substituents of a synthetic and biosynthetic amino acid residue side chains and each residue can have either D or L stereoconfiguration.

29 (Original). A macrocyclization method as in claim 28, wherein the substrate is sufficiently polar such that its solubility and that of the resulting macrocyclic molecule in the aqueous reaction medium is at least 0.1 g/L.

30 (Previously Presented). A macrocyclization method as in claim 28, wherein z is 6 to 24.

31 (Previously presented). A macrocyclization method as in claim 28, wherein the non-peptidic SPACER(s) comprises one or more of the following substituted groups such that the total number of atoms, z, in the linear backbone of the SPACER is greater than 6: C_{3-12}-alkyl, C_{3-12}-alkenyl, C_{3-12}-alkynyl, C_{3-7}cycloalkyl, C_{3-7}heteroalicyclic, aryl, heteroaryl, amine, C_{1-12}alkylamino, amide, ester, ketone, sulfoxide, ether, thioether, imine, sulfone.

32 (Previously Presented). A macrocyclization method as in claim 28, wherein the non-peptidic SPACER(s) comprises one or more of the following functional groups such that the total number of atoms, z, in the linear backbone of the SPACER is greater than 6: α,ω-alkandiyl, α,ω-alkane diol, α,ω-alkane diamine, ω-(1-alkanol)amine, ω-hydroxyalkanoate or ω-aminoalkanoate such that two or more functional groups are

linked by bonds chosen from the group of ether, amine, amide or ester bonds where each bond is independently chosen for each linkage.

33 (Previously Presented). A macrocyclization method as in claim 32, wherein the non-peptidic SPACER comprises one or more of the following functional groups linked together by either an amide or ester bond each bond being independently chosen at each occurrence: glycine, glycolate, O-(2-aminoethyl)glycolate, O-(2-ethanol)glycolate, O-(2-(2-aminoethoxy)ethyl)glycolate, O-(diethylene glycol)glycolate.

34 (Currently amended). A ~~macrocyclization~~ method for successive dimerization and macrocyclization of a peptide or modified peptide, the method comprising the steps of:

elongating a peptide or modified peptide substrate, which can not be cyclized by purified protein consisting of a TE domain from a polyketide synthase (PKS) or a non-ribosomal peptide synthetase (NRPS) ~~an excised TE domain protein~~, by contacting ~~excised~~ said TE domain ~~protein~~ with a first said peptide or modified peptide substrate under conditions conducive to formation of a TE-O-acyl substrate intermediate such that subsequently an intermolecular recognition element nucleophile from a second identical peptide or modified peptide substrate can displace the TE domain to form an elongated intermediate substrate homodimer;

repeating the elongating step until the elongated intermediate substrate oligomer is of sufficient length to undergo macrocyclization ~~catalyzed~~ by ~~excised~~ said TE domain ~~protein~~; and

allowing said elongated intermediate substrate to stay in the reaction media until the cyclization reaction is completed by ~~contacting the elongated substrate oligomer with excised TE under conditions conducive to formation of a TE-O-acyl substrate oligomer intermediate such that subsequently an intramolecular recognition element nucleophile can displace~~ the TE domain to form the macrocyclic product, thereby producing a macrocyclic peptide or modified peptide.

35 (Currently amended).A ~~macrocyclization~~ method as in claim 34, wherein the contacting of the ~~excised TE domain~~ protein with a substrate occurs in a medium that comprises at least 90 % water.

36 (Currently amended). A ~~macrocyclization~~ method as in claim 34, wherein the contacting of the ~~excised TE domain~~ protein with a substrate occurs in a medium that comprises at least 95 % water.

37 (Currently amended). A ~~macrocyclization~~ method as in claim 34, further comprising a non-water component(s) wherein the non-water component(s) is an organic solvent having a sulfoxide, ester, or amide functional group.

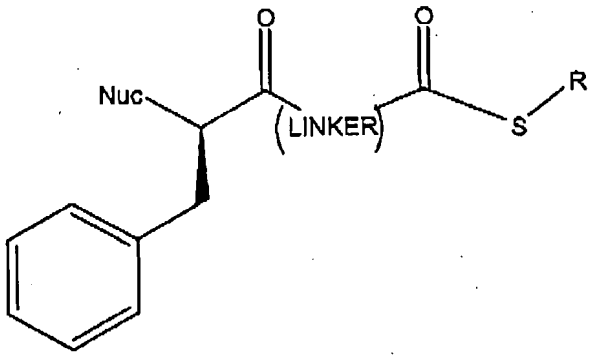
38 (Currently amended). A ~~macrocyclization~~ method as in claim 34, wherein the contacting of the ~~excised TE domain~~ protein with a substrate occurs in an aqueous solution comprising one or more buffers or other organic or inorganic salts.

39 (Currently amended). A ~~macrocyclization~~ method as in claim 34, wherein the pH of the reaction solution is in the range of 5 to 9.

40 (Currently amended). A ~~macrocyclization~~ method as in claim 39, wherein the pH of the reaction solution is in the range of 6 to 8.

41 (Currently amended). A ~~macrocyclization~~ method as in claim 39, wherein the pH of the reaction solution is 7.

42 (Currently amended). A ~~macrocyclization~~ method as in claim 34, with a substrate according to the formula:



wherein:

Nuc is chosen from NH_2 or OH;

LINKER is a group of atoms or functional group residues connecting the thioester and the 2-(Nuc)-3-phenyl-propionyl residue and LINKER comprises a linear not more than 14 atoms; and

R is N-C_{2-6}alkanoylC_{2-6}aminoalkyl group.

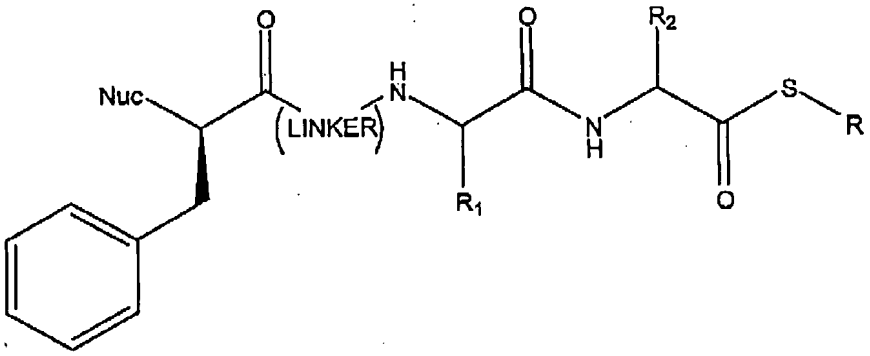
43 (Currently amended). A ~~macrocyclization~~ method as in claim 42, wherein the substrate is sufficiently polar such that its solubility and that of the resulting macrocyclic molecule in the aqueous reaction medium is at least 0.1 g/L.

44 (Currently amended). A ~~macrocyclization~~ method as in claim 42, wherein the substrate leaving group, SR, is N-acetylcysteamine (SNAC).

45 (Currently amended). A ~~macrocyclization~~ method as in claim 42, wherein the substrate Nuc is NH_2.

46 (Currently amended). A ~~macrocyclization~~ method as in claim 42, wherein the substrate Nuc is OH.

47 (Currently amended). A ~~macrocyclization~~ method as in claim 42, wherein the substrate can be represented by the formula:



wherein

Nuc is chosen from NH_2 or OH;

LINKER is a group of atoms or functional group residues connecting the thioester and the 2-(Nuc)-3-phenyl-propionyl residue, the LINKER comprises a linear backbone of not more that 9 atoms;

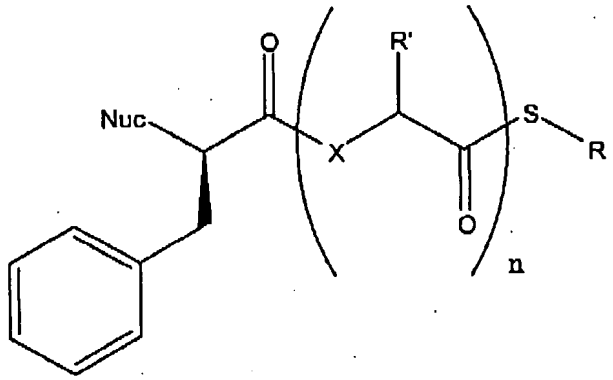
R is as defined for Claim 42; and

R_1 and R_2 are chosen from side chain substituents of the synthetic and biosynthetic amino acid residue side chains and each residue can have either D or L stereoconfiguration, R_1 and R_2 are chosen independently and can be the same or different.

48 (Currently amended). A ~~macrocyclization~~ method as in claim 47, wherein R_1 is a synthetic or biosynthetic amino acid residue side chain substituent including a substituted C_{1-6}aminoalkyl group.

49 (Currently amended). A ~~macrocyclization~~ method as in claim 48, wherein R_1 is L-3-aminopropyl.

50 (Currently amended). A ~~macrocyclization~~ method as in claim 42, wherein the substrate can be represented by the formula:



wherein:

R is as defined in Claim 42;

Nuc is chosen from NH_2 or OH;

n is an integral number greater than or equal to 5;

X is independently chosen for each occurrence of X from O and NH; and

R' is independently chosen for each occurrence for R' from the side chain substituents of the synthetic and biosynthetic amino acid residue side chains and each amino acid residue can have either D or L stereoconfiguration.

51 (Currently amended). A ~~macrocyclization~~ method as in claim 50, wherein:

Nuc is NH_2; and

X is NH for each occurrence of X in the substrate.

52 (Currently amended). A ~~macrocyclization~~ method as in claim 50, wherein:

Nuc is NH_2; and

X is chosen from O and NH for each occurrence of X in the substrate such that at least one occurrence of X in the substrate is O.

53 (Currently amended). A ~~macrocyclization~~ method as in claim 50, wherein:

Nuc is OH; and

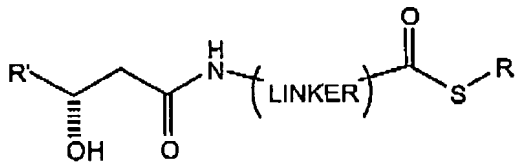
X is NH for each occurrence of X in the substrate.

54 (Canceled).

55 (Original). A method as in claim 50, wherein at least one occurrence of R' is 3-aminopropyl.

56-58 (Canceled).

59 (Previously presented). A macrocyclization method according to claim 1, wherein the substrate is represented by the formula:



wherein:

LINKER is a peptidic sequence, synthetic group comprising alkyl, cycloalkyl, alkenyl, alkynyl, aryl groups or a group that comprises a combination of two or more alkyl, cycloalkyl, alkenyl, alkynyl or aryl group regions and 0 to 3 heteroatoms selected from N, O, and S, or a combination thereof connecting the thioester and the 2-(Nuc)-3-phenyl-propionyl residue, the LINKER comprises a linear backbone of at least 14 atoms;

R is an optionally substituted C_{1-12} alkyl group; and

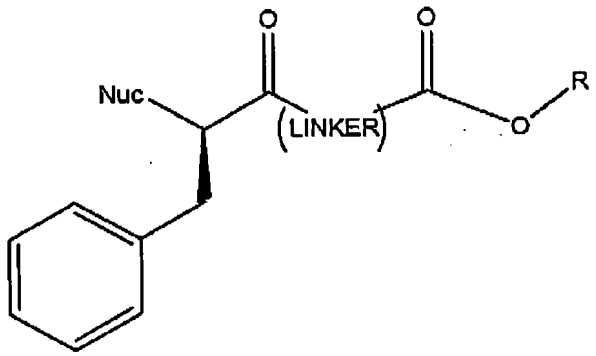
R' is a C_{1-18} alkyl group or a lipophilic group.

60 (Currently amended). A method for the preparation of macrocyclic molecules comprising:

contacting purified protein consisting of a TE domain from a polyketide synthase (PKS) or a non-ribosomal peptide synthetase (NRPS) ~~excised thioesterases (TE) domain protein~~ with a substrate that comprises an activated acyl residue and a pendant nucleophile separated by a linear backbone under conditions conducive to formation of a TE-O-acyl bond such that subsequently the pendant intramolecular nucleophile can displace the TE domain to form the macrocyclic product;

wherein the substrate can be represented by the formula A:

A.

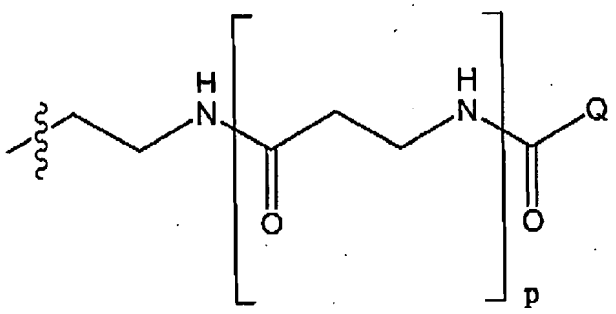


wherein

Nuc is chosen from NH_2, OH or SH;

LINKER is a peptidic sequence, a synthetic group comprising alkyl, cycloalkyl, alkenyl, alkynyl, aryl groups or a group that comprises a combination of two or more alkyl, cycloalkyl, alkenyl, alkynyl or aryl group regions and 0 to 3 heteroatoms selected from N, O, and S, or a combination thereof connecting the ester and the 2-(Nuc)-3-phenyl-propionyl residue, the LINKER comprises a linear backbone of at least 14 atoms; and

R is a group that can be represented by the formula:

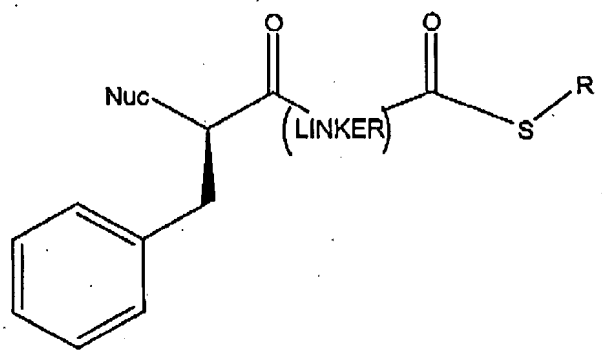


wherein Q is a group having between 4 carbon atoms and 20 carbon atoms and between 0 and 10 hetero atoms selected from N, O or S, which can optionally be tethered to a solid support, where each carbon of the linear backbone may be optionally substituted with 0, 1, or 2 groups selected from C_{1-6}alkyl, hydroxy, amino, halogen, C_{1-6}alkoxy, or oxo; and

p is an integer from 0 to 2; or

wherein the substrate can be represented by the formula B:

B.



wherein:

Nuc is chosen from NH_2, OH or SH;

LINKER is a peptidic sequence, a synthetic group comprising alkyl, cycloalkyl, alkenyl, alkynyl, aryl groups or a group that comprises a combination of two or more alkyl, cycloalkyl, alkenyl, alkynyl or aryl group regions and 0 to 3 heteroatoms selected from N, O, and S, or a combination thereof connecting the thioester and the 2-

(Nuc)-3-phenyl-propionyl residue, the LINKER comprises a linear backbone of at least 14 atoms; and

R is an optionally substituted C_{1-12} alkyl group or an optionally substituted N-C_{2-6}alkanoyl-C_{2-6}aminoalkyl group.